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Commentary

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Use of the A_{2A} adenosine receptor as a physiological immunosuppressor and to engineer inflammation *in vivo*

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Abstract

Inflammation must be inhibited in order to treat, e.g., sepsis or autoimmune diseases or must be selectively enhanced to improve, for example, immunotherapies of tumors or the development of vaccines. Predictable enhancement of inflammation depends upon the knowledge of the ''natural'' pathways by which it is down-regulated *in vivo*. Extracellular adenosine and A_{2A} adenosine (purinergic) receptors were identified recently as anti-inflammatory signals and as sensors of excessive inflammatory tissue damage, respectively (Ohta A and Sitkovsky M, Nature 2001;414:916–20). These molecules may function as an important part of a physiological ''metabolic switch'' mechanism, whereby the inflammatory stimuli-produced local tissue damage and hypoxia cause adenosine accumulation and signaling through cyclic AMP-elevating A_{2A} adenosine receptors in a delayed negative feedback manner. Patterns of A_{2A} receptor expression are activation- and differentiation-dependent, thereby allowing for the ''acquisition'' of an immunosuppressive ''OFF button'' and creation of a time-window for immunomodulation. Identification of A_{2A} adenosine receptors as ''natural'' brakes of inflammation provided a useful framework for understanding how tissues regulate inflammation and how to enhance or decrease (engineer) inflammation by targeting this endogenous anti-inflammatory pathway. These findings point to the need of more detailed testing of anti-inflammatory agonists of A_{2A} receptors and create a previously unrecognized strategy to enhance inflammation and targeted tissue damage by using antagonists of A_{2A} receptors. It is important to further identify the contributions of different types of immune cells at different stages of the inflammatory processes in different tissues to enable the ''tailored'' treatments with drugs that modulate the signaling through A_{2A} purinergic receptors.

Keywords: Inflammation; Adenosine; Engineering; Cytokines; Tissue damage; Cancer immunotherapy

1. Introduction

The term ''bioengineering'' has been applied mostly to the design and synthesis of biologically active molecules and to tissue engineering [1,2]. We propose to define the term ''engineering inflammation'' to denote the manipulation of the intensity and the time-course of inflammatory processes *in vivo*.

Host defense-initiated inflammatory processes are crucial for the destruction of pathogens and virus-infected cells by activated immune cells, which secrete a variety of pro-inflammatory cytokines and cytotoxic molecules [3]. However, the action of toxic pro-inflammatory molecules also results in undesirable outcomes, mostly due to collateral tissue damage. It is well accepted that prolonged and/or inappropriate inflammation contributes to the pathogenesis of many diseases including cancer, heart disease, and atherosclerosis [4–8]. The extensive development of anti-inflammatory drugs reflects the clinical desirability of preventing excessive tissue damage and the need to better understand the processes that control and terminate inflammation. This is also the driving force behind the recent intensification of efforts to understand anti-inflammatory mechanisms in different diseases [9,10].

We will discuss the less extensively studied issues of *enhancement* of inflammation and targeted local tissue damage, since addressing these issues promotes both better understanding of the fundamental mechanisms of the immune response and forms the basis for predictable inhibition or enhancement of the immune response, i.e. engineering inflammation. This review describes the recently discovered physiological immunosuppressive

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Abbreviations: ADA, adenosine deaminase; cAMP, cyclic adenosine monophosphate; CTL, cytotoxic T lymphocytes; IFN-γ, interferon-γ; IL, interleukin; LPS, lipopolysaccharide; SCID, severe combined immunodeficiency; TCR, T-cell receptor; and TNF-α, tumor necrosis factor-α.

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loop where different pro-inflammatory stimuli disturb the local tissue environment due to a diminished blood supply, local tissue hypoxia, and accumulation of extracellular adenosine. This triggers immunosuppressive cAMP accumulation in immune cells via signaling through A_{2A} adenosine receptors. The A_{2A} adenosine receptor signaling suppresses inflammation in a delayed, negative feedback manner because of inhibition by cAMP of molecular intermediates of pro-inflammatory signaling pathways [11]. It is expected that the discovery of the adenosine receptor-based natural anti-inflammatory pathway should re-invigorate interest in adenosine-based compounds, which have been designed and synthesized to modulate immunosuppressive adenosine receptors [12–17]. Appropriate A_{2A} adenosine receptor agonists and antagonists have promise for the engineering of inflammatory processes *in vivo* by selectively utilizing or disengaging the natural, endogenous anti-inflammatory pathway.

2. Need to identify endogenous anti-inflammatory pathways

Manipulation of inflammatory processes may include not only efforts to inhibit inflammation, but also the development of approaches to enhance local inflammatory processes. This is illustrated by the desirability to develop better adjuvants for vaccine development [18] and stronger pro-inflammatory local tissue damage during immunotherapies of cancerous tumors [19]. Accordingly, a current goal of pharmacological research is to develop both pro-inflammatory and anti-inflammatory drugs. This should be done with the understanding that the pharmacological agent can be a useful anti-inflammatory drug even if it targets a pathway that is not an important part of a physiological mechanism, but the rational development of valuable pro-inflammatory agents will require an improved understanding of natural inhibitors of inflammation. A rather obvious option is to enhance inflammatory processes simply by increasing the dose of inflammatory stimuli, but this may be accompanied by unacceptable side-effects. It appears that the least dangerous approach to enhancing inflammation may lie in antagonizing the physiological mechanisms that attenuate it *in vivo*, but this has not been possible.

Thus, the efforts to reveal the endogenous mechanisms that terminate inflammation and thereby protect tissues from excessive damage may be richly rewarded by identification of better targets for immunomodulation and by providing previously unavailable options of enhancing inflammation through the use of novel drugs that antagonize natural anti-inflammatory processes and result in persistence of pro-inflammatory molecules in targeted local tissue environments. In addition, the identification of endogenous inhibitors of inflammation is expected to improve the design and development of novel inflammation-inhibiting drugs that will be able to recruit the ‘‘natural’’ pathway.

3. “Metabolic switch” hypothesis of extracellular accumulation of molecular “signals” of excessive tissue damage

One of the promising approaches to identifying endogenous anti-inflammatory molecules is to determine whether any of the numerous molecules that are capable of blocking inflammation [3] are actually involved in the physiological mechanism that ‘‘senses’’ excessive tissue damage and triggers the inhibition of secretion of pro-inflammatory molecules. Our studies were based on the assumption of the existence of a ‘‘metabolic switch’’, which is turned on at some ‘‘unacceptable’’ threshold of collateral tissue damage. Indeed, the tissue damage-associated interruptions in blood supply are expected to result in local tissue hypoxia and in appropriate adaptive changes in cell metabolism. These changes may include qualitatively and quantitatively different ‘‘repertoires’’ of cellular metabolites that could be transported into the extracellular environment and trigger inflammation-inhibiting signaling through their receptors on immune cells. These considerations postulate an immunosuppressive function for a ‘‘non-professional’’ anti-inflammatory molecule(s). Such molecules may be accumulated as the outcome of adaptive metabolic changes to hypoxia in injured tissues that ensure both cell survival and reporting of the ‘‘excessive’’ tissue damage. This, in turn, will terminate an inflammatory response by triggering immunosuppressive signaling. We considered extracellular adenosine to be an attractive candidate for such a reporter and focused our studies on testing the possible role of adenosine as an endogenous immunosuppressive molecule.

4. Considerations of a possible role of cAMP-elevating extracellular adenosine and adenosine receptors in the regulation of inflammatory processes

4.1. Sources of extracellular adenosine

It has been known for a long time that inflammatory tissue damage is accompanied by accumulation of extracellular adenosine in inflamed areas due to its release from non-immune and immune cells [20–29]. Also contributing to the accumulation of adenosine is the release of rapidly metabolized ADP and ATP from various cells including platelets, mast cells, and endothelial cells [30]. Local tissue hypoxia in inflamed areas represents one of the most important conditions leading to adenosine release and accumulation [31–33], and isolated heart studies suggest that adenosine kinase inhibition in hypoxic conditions is primarily responsible for adenosine accumulation in sites of local tissue injury because about 80% of adenosine is rephosphorylated to AMP by this enzyme [34] in normoxic myocardium. Hypoxic inhibition of adenosine kinase leads

to a 15- to 20-fold increase in intracellular and extracellular adenosine [34].

Extracellular adenosine may also accumulate due to a deficiency in ADA in patients with SCID [35] and to changes in adenosine metabolism induced by some pharmacological agents [36–38].

4.2. The history of extracellular adenosine and adenosine receptor studies

Investigations during more than three decades of the effects of adenosine on inflammatory processes and of the immunosuppressive properties of adenosine-induced, cAMP-elevating, extracellular receptor-mediated signaling can be divided into three main periods. The first two decades (i) provided the first demonstrations of inhibition by extracellular adenosine of cells involved in the inflammation processes, (ii) implicated the cAMP-elevating pathway and adenosine (purinergic) receptors as regulators of inflammation, and (iii) revealed multiple stimuli and conditions that induce adenosine release from different cells into extracellular space [20–26,31,39–44]. This period encompasses the development of the "purinergic" receptors concept by Burnstock [45] that led to the foundation of the intellectual framework for later experiments to consolidate results obtained in different biological disciplines and stimulate pharmacological research.

The next two decades provided superior investigative tools through the synthesis of novel ligands [46] and molecular cloning and characterization of four subtypes of adenosine receptors [30,47–49]. The interest of pharmacologists in adenosine receptors has been heightened dramatically by the findings of Cronstein [36], Fredholm and coauthors [38] and others [37] that important anti-inflammatory drugs such as methothrexate and certain salicylates exert their effects by utilizing endogenous adenosine-mediated signaling. Pharmacologically induced extracellular adenosine has also been implicated in the anti-inflammatory action of nimesulide [37] and the neuroprotective drug propentofylline [38].

The actions of extracellular adenosine were explained by signaling through G protein-coupled receptors that are subdivided into A_1, A_{2A}, A_{2B}, and A_3 subtypes. A_1 and A_3 receptors are coupled to G_o and G_i proteins, and A_{2A} and A_{2B} receptors interact with G_s proteins leading to intracellular cAMP accumulation [48]. A_{2B} receptors also couple to G_q in some cells [50].

The effects of adenosine in different tissues may depend upon the repertoire of adenosine receptors present on the cell surface. Patterns of A_{2A} adenosine receptor expression are activation- and differentiation-dependent, thereby allowing for the "acquisition" of an immunosuppressive "OFF button" and creation of a time-window for immunomodulation. This could be the general property of purine receptors as shown in studies of both ATP and adenosine receptors [51–54].

Different receptors may have opposite effects on the same function. It was shown that the same process (neutrophil adherence to endothelium) could be inhibited by A_2 receptors and enhanced by A_1 receptors [55]. Detailed studies of the effects of adenosine on different immune cells [36,56–64] provided important clues as to what the possible roles of extracellular adenosine could be *in vivo*.

Indeed, it is now well accepted that the activation of A_{2A} receptors on lymphoid cells stimulates an anti-inflammatory response [36,57,59,60,62,63]. T cells were shown to express immunosuppressive cAMP-elevating A_{2A} adenosine receptors, which are capable of blocking TCR-triggered proliferation, granule exocytosis, FasL expression, and secretion of cytokines such as IFN-γ [42,62,63]. Similarly, adenosine receptors that inhibit immune functions upon activation were demonstrated on human basophils [43]. In contrast, A_3 receptors have more complex effects as they inhibit eosinophil migration and trigger degranulation of rodent mast cells [27,30,65,66].

Of special interest in the context of this commentary are the documented effects of adenosine on the secretion of pro-inflammatory cytokines, e.g. IL-12, TNF-α, and others, by monocytes and macrophages [58,59,67–72]. Agonists of both A_{2A} or A_3 receptors were shown to block activation of macrophages, but the relative contribution of these receptors is yet to be demonstrated conclusively. Indeed, some contradictory conclusions were reached in implicating the A_{2A} versus A_3 receptors in these processes. For example, McWhinney *et al.* [70] and Sajjadi *et al.* [71] suggest that the adenosine analog N^6-(3-iodobenzyl)adenosine-5′-*N*-methyluronamide (IB-MECA)-mediated inhibition of LPS-induced TNF-α secretion was mediated by A_3, but not by A_{2A}, receptors, but Link *et al.* [72] and Bouma *et al.* [68] reported that inhibition of LPS-induced IL-12 secretion by human monocytes was mediated by A_{2A}, but not A_1 or A_3 receptors. A possible explanation for this discrepancy may lie in the observation that the compounds IB-MECA and Cl-IB-MECA, which have been used as selective A_3 agonists, are poorly selective over G_s protein-coupled A_{2A} adenosine receptors [13].

Thus, while pharmacological studies offered important clues, the conclusive identification of individual adenosine receptors in mediating the effects of adenosine and adenosine analogues, awaited genetic *in vivo* experiments. Such genetic studies have been greatly facilitated by the development of mice that are deficient in genes for A_{2A} [73,74] or A_3 adenosine receptors [75].

The development of adenosine receptor gene-deficient mouse models extended the purine field into the current, third stage of studies in which the major goal is to understand the *in vivo* physiological roles of adenosine receptor-mediated signaling in immune responses and in the pathogenesis of inflammatory diseases. The use of A_{2A} receptor-deficient mice provided the first conclusive *in vivo* evidence of A_{2A} receptors playing a critical role in the down-regulation of acute inflammation [11].

5. Genetic *in vivo* evidence for the physiological mechanism of attenuation of inflammation and protection from excessive tissue damage by extracellular adenosine A_{2A} receptors

We began our studies by assuming that tissue damage-associated hypoxia results in extracellular accumulation of metabolic intermediates that trigger intracellular cAMP increases, which, in turn, inhibit inflammatory cells [76,77]. Accordingly, the identification of cAMP-elevating receptors and ligands, among a great number of G_s-coupled receptors [78], which may function as endogenous inhibitors of inflammation, became a goal of subsequent experiments. It has been reported that different cAMP-elevating ligands including catecholamines, prostaglandins, dopamine, and histamine, as well as extracellular adenosine and other anti-inflammatory molecules, have immunosuppressive pharmacological properties. These have been considered among many other molecular candidates as potential anti-inflammatory stimuli *in vivo* [9,79–81].

The focus on cAMP-elevating adenosine A_{2A} receptors in our laboratory was due to the convergence of long-term studies of cAMP as an OFF mechanism in T-cell function [82–84] and studies of Ca^{2+} signaling in lymphocytes [85,86]. These studies led to the prediction of the existence of a second mechanism of cytotoxicity [82] and to considerations of cell-permeabilizing extracellular ATP in lethal hit delivery by CTL [87,88], as well as to the evaluation of the contribution of receptors for extracellular nucleotides such as ATP and adenosine in regulating the immune response [61,63]. Subsequent studies of extracellular purines, ATP, and adenosine, and "purinergic" receptors [80,89,90] in T-cell differentiation, lethal hit delivery, and other effector functions [28,61–64,87] were related mostly to $P2_{y1}$ and $P2_{x7}$ subtypes of ATP receptors and A_{2A} adenosine receptors. It was shown that G protein-coupled $P2Y_2$ ATP receptors are expressed as immediate early response genes in T cells [53], and that ATP-gated membrane channels and pore-forming P_{2z} (now designated $P2_{x7}$ receptors) are expressed in a T-cell differentiation-dependent manner [51,61]. The role of ATP-gated $P2_{x7}$ receptors in lethal hit delivery was excluded by a direct comparison of requirements for the pore-forming ATP^{4-} complex and the CTL-mediated lethal hit delivery to targets [91]. A physiologically important role of these ATP receptors in T cells or any immune cell *in vivo* remains to be firmly established.

Investigations of the effects of extracellular adenosine have revealed that these physiologically abundant molecules trigger powerful immunosuppressive, cAMP-mediated responses by virtue of signaling through A_{2A} adenosine receptors in T cells [62–64,92–94]. It was shown that extracellular adenosine and the A_{2A} adenosine receptor agonist CGS21680 [2-*p*-(2-carbonylethyl)-phenylethylamino-5′-*N*-ethylcarboxamidoadenosine] inhibits TCR-triggered IL-2 receptor up-regulation, thereby explaining the inhibition of T-cell proliferation by extracellular adenosine [62]. Adenosine was shown to be capable of directly inducing apoptosis by elevating cAMP in a small (about 10% of total thymocytes) subset of immature thymocytes, which was interpreted as an indication of its possible role in the regulation of thymocyte differentiation [61,93]. Paradoxically, adenosine has been also shown to provide a TCR-inhibiting signal to immature double-positive $CD4^+CD8^+$ thymocytes, thereby rescuing these cells from antigenic peptide-triggered apoptosis and leading to the suggestion of a role of adenosine in regulating the positive and negative selection of thymocytes [95]. In addition, an analysis of the effects of extracellular adenosine on TCR-triggered thymocyte apoptosis in ADA deficiency using genetically engineered ADA-deficient mice has provided support for the hypothesis that adenosine-mediated signaling contributes to ADA SCID-associated depletion of T cells [95].

Since the injection of A_{2A} agonists did prevent damage in tissue injury models in kidney [96], heart [97], lung [98], skin [99], vascular smooth muscle [5], and spinal cord [17], it was concluded that pharmacologically activated A_{2A} receptors are at least capable of being anti-inflammatory in these models. Indeed, in their totality, the pharmacological data accumulated over almost 30 years are consistent with a potential role of cAMP-elevating adenosine receptors to attenuate inflammation *in vivo*. However, this notion has not been widely accepted by investigators outside this field since adenosine receptors are not the only candidates for this role. Activation of some other G_s protein-coupled cAMP-elevating receptors (including histamine, dopamine, prostaglandin, and β_2 adrenergic receptors) can also inhibit inflammatory processes *in vivo*, which led to their consideration as potential physiological OFF signals [79,81,100,101]. Activation of both A_{2A} and A_{2B} receptors is capable of increasing cAMP levels in immune cells, and both receptors had to be considered as potentially immunosuppressive *in vivo*. In addition, putative activation of G_i-coupled A_3 adenosine receptors with IB-MECA also was reported to inhibit acute inflammation [70,71], and this pointed to the need to investigate the A_3 receptor as an endogenous immunosuppressor.

It has become clear that while pharmacological studies alone provide information as to what adenosine receptors are potentially capable of doing, no evidence existed to support a physiological role for adenosine receptors in the immune response. The use of A_{2A} adenosine receptor-deficient mice in preliminary phenotype-screening assays has not been helpful either, since no significant differences between $A_{2A}R^{+/+}$ and $A_{2A}R^{-/-}$ mice were detected in terms of total cell numbers or cell surface markers (data not shown).

The first strong evidence for the critical role of A_{2A} receptors in the regulation of inflammation *in vivo* was provided by testing the prediction that the absence of A_{2A} receptors would lead to enhanced inflammation and

increased tissue damage. This was done using A_{2A} receptor-expressing and A_{2A} receptor gene-deficient mice in models of acute liver inflammation and sepsis [11]. It was confirmed that A_{2A} receptor-deficient mice represent an excellent model to answer the questions posed since these mice do not express functional A_{2A} receptors, but do express other candidate cAMP-elevating receptors (e.g. β adrenergic and prostaglandin receptors), which have been considered as alternative candidates for a role in physiological anti-inflammatory pathways [11].

The most likely involvement of A_{2A} receptors was expected in conjunction with severe tissue damage. This, in turn, prompted the choice of short-term fulminant hepatitis and sepsis as *in vivo* models to be first tested in wild-type and A_{2A} receptor gene-deficient mice. Endotoxin-induced sepsis, carbon tetrachloride, and *Pseudomonas aeruginosa* exotoxin A-mediated hepatotoxicity, as well the infected wound model (air-pouch), were utilized in these studies.

Subsequent assays of T-cell-, macrophage- and cytokine-dependent tissue injury in A_{2A} adenosine receptor-deficient mice established dramatically increased local tissue damage and the prolonged presence of pro-inflammatory cytokines such as TNF-α, IFN-γ, and IL-12 [11] in these mice, but not in wild-type mice. It was shown that sub-threshold doses of inflammatory stimuli, which do not cause liver damage in wild-type mice, do induce extensive liver damage and sustained elevated levels of cytokines in mice deficient in A_{2A} receptor expression. A similar outcome was observed when A_{2A} receptors were blocked pharmacologically. The injection of the A_2 receptor antagonist ZM241385 [4-(2-[7-amino-2-(2-furyl)-[1,2,4]triazolo[2,3-*a*][1,3]triazinyl-amino]ethyl)-phenol] into wild-type mice also resulted in exacerbated tissue damage in response to sub-threshold doses of inflammatory stimuli [11].

These data provided the first direct and conclusive evidence for an *in vivo* role by A_{2A} adenosine receptors in the regulation of inflammation, and pointed to A_{2A} adenosine receptors as critical in playing a non-redundant role in the down-regulation of inflammation *in vivo*. The A_{2A} receptors seem to be non-redundant because of the failure of all other anti-inflammatory mechanisms to compensate for the absence of A_{2A} receptors in preventing dramatic tissue damage. However, it is expected that when the intensity of inflammatory stimuli is not as high, there is a coordinated functioning of several different anti-inflammatory mechanisms, including the possible involvement of other adenosine receptors and of other important anti-inflammatory molecules [9].

It is interesting to note that A_{2A} receptor-deficient mice have been reported to be protected from ischemic brain injury [74], suggesting that these receptors may play an opposite role in the brain as compared with peripheral tissues.

The idea of extracellular adenosine as an endogeneous immunoregulator and an OFF switch of inflammation is supported by: (i) the abundance of this ubiqutous signaling metabolite, (ii) immunosuppressive properties of adenosine, and (iii) unique capabilities of adenosine to reflect changes in the physical and biochemical environment, particularly in inflamed areas.

6. Engineering inflammation

Identification of A_{2A} adenosine receptors as "natural" brakes of inflammation provided a useful framework for understanding how tissues regulate inflammation and how to enhance or decrease (engineer) inflammation by targeting the endogenous anti-inflammatory pathway. This provides additional justification for the vigorous development of anti-inflammatory agonists of A_{2A} receptors and creates a previously unrecognized strategy to enhance inflammation and targeted tissue damage by using antagonists of A_{2A} receptors. It is important now to further identify the contributions of different types of immune cells at different stages of inflammatory processes in different tissues to enable the "tailored" treatments with novel drugs that modulate the A_{2A} purinergic receptor-mediated signaling.

6.1. Inhibition of inflammation

Although endogeneous adenosine that accumulates in tissues has been demonstrated to protect tissues under some circumstances, far greater protection can be elicited by synthetic compounds such as the non-selective A_1/A_{2A} agonist AMP 570 [102], or by a widely used selective agonist of A_{2A} receptors, CGS21680 [103]. Recently, Linden and co-authors [104] described and characterized a very promising A_{2A} adenosine receptor agonist, ATL146e, with far greater potency and selectivity than CGS21680. This agonist has been shown to markedly reduce ischemia–reperfusion or traumatic injury and neutrophil infiltration in many tissues including kidney [105], skin [99], lung [98], blood vessels [5], and spinal cord [17].

Selective A_{2A} receptor agonists may be more effective than endogenous adenosine as inhibitors of inflammation for two reasons. First, adenosine and its breakdown product, inosine, may activate pro-inflammatory pathways that are mediated by A_1 [106] or A_3 receptors [107]. Second, optimal effectiveness of A_{2A} receptor activation probably requires the triggering of receptors on circulating leukocytes, lymphocytes, platelets, and/or vascular endothelium. In this sense, synthetic compounds are much more effective at activating receptors in blood than adenosine, which is rapidly metabolized in blood [108].

Thus, results of these preliminary animal studies point to the promising uses of A_{2A} receptor agonists as therapeutic agents to treat ischemic, infection-associated, and trauma-associated inflammation provided that possible side-effects are taken care of by designing more

tissue-specific treatments or using a ''neoceptor/neoligand'' approach [15] when it is feasible.

6.2. Enhancement of inflammation

The demonstration of a critical role of extracellular adenosine A_{2A} receptors in the down-regulation of inflammation *in vivo* represents the first example that physiological, metabolic changes in the inflamed local tissue environment can serve as ''reporters'' of excessive tissue damage that can provide a signal to terminate inflammatory processes. This immunosuppressive mechanism functions by taking advantage of very fundamental cellular processes where physiologically abundant and ubiquitous molecules of adenosine can be produced by virtually all cells, resulting in a negative feedback loop to inhibit inflammation-producing immune cells and protect surrounding tissues.

7. Perspectives

An optimistic prognosis of current, third stage studies of purinergic receptors includes implementation of promising strategies to engineer inflammation in order to afford, for example, protection of transplanted tissue from the immune system, without compromising immune protection against infection and immunotherapy for cancer. These goals will require significant progress in efforts to selectively target the adenosine receptors and in the development of adenosine receptor ligands [16,17]. The use of such drugs may be facilitated by the development of promising partners for agonists of adenosine receptors that produce synergistic inhibition of the immune system, e.g. type IV phosphodiesterase inhibitors [109]. Possible side-effects of such ligands could be dealt with by employing the recently described, elegant ''neoceptor/neoligand'' concept [15].

Only the A_{2A} receptors have been convincingly implicated so far as participating in natural anti-inflammatory mechanisms. The role of other adenosine receptors remains to be firmly established. It is important to reconcile complicated effects of G_i protein-coupled A_3 adenosine receptors, which have been suggested to possess anti-inflammatory properties, when activated with IB-MECA pharmacologically [71], but which also trigger pro-inflammatory events in mast cells [110].

Indeed, extracellular adenosine may have strong pro-inflammatory effects, as evidenced by adenosine-mediated enhancement of neutrophil-mediated ischemic tissue damage [111]. It is most likely that adenosine plays both pro-inflammatory and anti-inflammatory functions, depending on which immune cells and cytokines are involved in particular inflammatory processes and disease. The careful investigation of recruitment of different classes of adenosine receptors on different immune cells in future studies may help to reconcile pro-inflammatory and anti-inflammatory properties of extracellular adenosine and the roles played by different adenosine receptors.

The genetic evidence implicating A_{2A} adenosine receptors in a non-redundant physiological mechanism to terminate inflammation *in vivo* during the course of acute inflammation in liver disease and sepsis is expected to result in more efforts to develop therapeutic strategies where these receptors are utilized as attractive natural, pharmacological targets for anti-inflammatory drugs. Recent progress in the design, synthesis, and analysis of adenosine-based ligands [12–17] suggests that these pharmacological challenges will be met rather successfully [15]. These considerations point to the relative paucity in selective A_{2A} receptor antagonists. This seems to be a promising venue of future medicinal chemistry and pharmacological research to develop selective and highly effective antagonists of A_{2A} adenosine receptors.

The pharmacological targeting of purine receptors requires further studies of the role of other individual adenosine and ATP receptors in different tissues at different stages of the inflammatory processes. Accordingly, individual disease models should be characterized to answer the following questions: First, which inflammatory stimuli and which pro-inflammatory cytokines mediate pathogenesis of the disease in each individual tissue? Second, is there significant tissue damage associated with the inflammatory processes? Third, what are the types of cells that are targeted by pro-inflammatory molecules? Fourth, which immune cells produce these pro-inflammatory molecules? Fifth, what is the repertoire of purinergic receptors on these immune cells? Sixth, what are the other molecules that coordinate the anti-inflammatory response? The acute inflammation models that were used to uncover the role of A_{2A} receptors [11] represent only an important first step that should be followed by detailed studies of the role of adenosine receptors on chronic inflammation models and in T-cell-mediated immune processes.

Having this information will greatly facilitate attempts to engineer inflammation in novel clinical protocols. However, additional basic knowledge of the mechanisms leading to the accumulation of extracellular adenosine is still required. Indeed, the accumulation of adenosine in inflamed hypoxic areas is best explained by hypoxia-associated inhibition of adenosine kinase as suggested by Schrader and co-authors [34]. Their calculations suggest that even minor hypoxia-associated increases in the concentration of intracellular AMP will be translated into large increases in adenosine concentration.

The molecular mechanisms of inhibition of adenosine kinase need to be clarified, and it seems promising to consider the possibility that the accumulation of adenosine in hypoxic tissues may be due, at least partially, to the activities of hypoxia-inducible factors [112], which are stabilized and are expressed and active in hypoxic and/or in activated T cells as immediate early response gene pro-

ducts [113]. Because the major function of HIF is to switch cells to glycolysis, it is possible to test the hypothesis that HIF-1 is involved in metabolic changes that lead to extracellular adenosine accumulation.

Thus, the current stage of inflammation and adenosine studies is characterized not only by utilization of the most advanced genetic and molecular techniques to manipulate adenosine receptor signaling in different disease models, but also by the need to better understand issues of adenosine metabolism that have been the focus of classical biochemical investigations.

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